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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. — No. 195.

THE SENSORY REACTIONS OF AMPHIOXUS.

BY G. H. PARKER.

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By G. H. Parker.

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1. Introduction.

Whatever position may be assigned to amphioxus in the classification of the chordates, it is now generally admitted that this animal retains many of the more primitive features of the ancestors of the vertebrates. Such features not only occur in its anatomy and embryology, but are to be expected in its activities. As the structure of amphioxus throws light on the complex organization of the vertebrates, so its activities may give some indication of the way in which the more complex functions of these animals have come into being. It is from this standpoint that I have undertaken the study of the sensory reactions of amphioxus.

The material upon which this work was based is the so-called West Indian amphioxus or lancelet, Branchiostoma caribbaeum Sundevall, a close relative of the common European form, B. lanceolatum (Pallas). This material was collected and studied during the summer of 1905 while I was at the laboratory of the Bermuda Biological Station located at Hotel Frascati, Flatts Village, Bermuda. The living

[1] Contributions from the Bermuda Biological Station for Research, No. 12.

lancelets were obtained from the Flatts Inlet, which leads from the outer waters to Harrington Sound. This inlet, through which a strong tidal current is almost always running in one direction or the other, contains long stretches of coarse coral and shell sand, and it was in these sandy stretches, especially near the open mouth of the inlet, that the lancelets were found in abundance. They likewise occurred, as recorded by Barbour (:05, p. 110), in the sandspit near the inner end of the inlet opposite Hotel Frascati, but they were by no means so abundant there as in the coarse shelly stretches which were near the outer mouth of the inlet and at low tide were still covered by several feet of water. From this source, with the assistance of some of the negro boys from the neighborhood, a daily supply of large, vigorous lancelets was obtained, and, as the animals were available in the laboratory almost immediately after they were caught, the conditions were unusually favorable for a study of their sensory reactions. For experimental purposes these lancelets proved to be very satisfactory. They could be kept for a number of days in a vigorous condition in large glass jars containing sea water and some coral sand, provided that from time to time the sea water was renewed, and their resistance to the adverse conditions of operative experiments was as great as that of B. lanceolatum (Haeckel, '80, p. 141).

In the shoal water of Harrington Sound northwest of Trunk Island the expeditions from the laboratory on several occasions dredged Andrew's lancelet, Asymmetron lucayanum Andrews, but this species was not sufficiently accessible nor abundant to make it a satisfactory form for experimentation. In testing the sensory reactions of the lancelets I therefore limited my work to the more common species, Branchiostoma caribbaeum, and attempted to determine the reactions of this species to light, to heat, and to mechanical and chemical stimuli.

2. Light.

Although the sensitiveness of amphioxus to light was known to Costa ('39, p. 4)[2] and many other earlier investigators, and has since been generally admitted, much difference of opinion has been expressed as to the degree of this sensitiveness. Willey ('94, p. 10) declares that "if a lighted candle is carried into a dark room in which amphioxus are being kept in glass jars, the excitement produced among the small

[2] The statements concerning the reactions of amphioxus to light given by Costa do not occur in his first account of this animal (Costa, '34, p. 49) as cited by Krause ('97, p. 513), but in his later and more lengthy description (Costa, '39, p. 4).

fish is indescribable," and Nagel ('96, p. 79) states that "plötzliche Belichtung lässt dann die sämtlichen Exemplare wild durchs Wasser jagen." Hesse ('98[b], p. 461) confirms these observations and records that light calls forth vigorous swimming. On the other hand, Nüsslin ('77, p. 23), who also tried sudden illumination, affirms that amphioxus is only very slightly sensitive to light, and Rohon ('82, p. 38) expresses the belief based on experimental evidence that the so-called light reactions of this animal are really reactions to heat, and that it is not sensitive to light at all, or at most only to a very slight degree, — an opinion concurred in by Kohl ('90, p. 185).

In consequence of this difference of opinion the first question to be settled was, whether amphioxus was or was not sensitive to light. I therefore repeated the experiments made by Willey, Nagel, and Hesse, and with confirmatory results. When sunlight, daylight, lamplight, or even candle-light was allowed to fall into a previously darkened glass dish containing a dozen or more amphioxus, the whole company swam about for a minute or so in wild confusion and then dropped as though exhausted to the bottom. At first sight this seemed to be conclusive evidence of the great sensitiveness of amphioxus to light, but a more careful scrutiny of the steps in the experiment showed that this was not necessarily so. When light first fell upon the dish, all the lancelets did not begin at once to swim about excitedly. What usually happened was that a few moved slightly, and in doing so they touched others; these then sprang suddenly into active locomotion, and in an instant the whole assembly was swimming in wild confusion. Thus it would seem that, while light was the initial stimulus for a few individuals, the wild and excited swimming which gave the impression of great sensitiveness to light was not due directly to this factor, but to mechanical stimulation caused by mutual contact.

To test this hypothesis I placed a shallow dish of sea water containing twenty live amphioxus in a dark room and, after about an hour, I threw upon it the light of a strong lamp; in a few seconds all the animals were swimming as though in the utmost excitement. I then let them rest in the dark for a full hour, whereupon, without illuminating the dish, I felt for one with a glass rod, and, having touched it, I soon heard an agitated movement in the dish such as had followed the previous sudden illumination. Upon turning on the light the animals were found to be in as much commotion as at the trial in which light had been the initial stimulus. I then took the twenty animals that had been used in these two experiments and put each one in a separate dish of sea water and placed each dish in an approximately light-proof compartment by itself. After an hour I

illuminated dish by dish in turn with the same lamp that had caused the whole assembly of lancelets to swim wildly about when together, and noted the individual reactions. Of the twenty animals tested, twelve reacted, some more, some less, but none vigorously; eight absolutely failed to give any response whatsoever, even after continued illumination. The twenty animals were then placed together in a single glass dish, and, after about an hour, they were suddenly subjected to bright illumination, with the result that they exhibited the same commotion as was seen in the first of these experiments. I therefore conclude that the wild swimming recorded by Willey, Nagel, and Hesse is not, as they believed, evidence of great sensitiveness to light, but is the result of the mechanical stimulation of one amphioxus touching another, and that amphioxus, as stated by Nüsslin, is really only very slightly sensitive to light.

Rohon's belief that the so-called light reactions of amphioxus are really reactions to radiant heat is not supported by my observations. Contrary to the statements of Rohon, amphioxus is responsive to light that has passed through a heat screen; nor does Rohon seem to have been aware of the fact, pointed out later by Krause ('97, p. 514), that a few centimeters of sea water is as effective a heat screen as the alum solution that he used, and that consequently in all his experiments that were carried on with some depth of sea water, the animals that were supposed to be subjected to radiant heat were as a matter of fact as completely shielded from it as though they were behind an alum screen. Kohl's concurrence in Rohon's opinion does not seem to be founded on any observations of his own, for he ('90, p. 182) states that he had no opportunity to work with living material. I therefore believe that the slight initial locomotor response that amphioxus usually makes when a beam of light is suddenly thrown on it is dependent upon the light waves themselves and not upon radiant heat.

Although amphioxus is assuredly not so sensitive to light as many investigators have supposed it to be, it does show a capacity to respond to a considerable range of this form of stimulus. Nagel ('96, p. 80) stated that its characteristic reactions could often be called forth by a relatively weak stimulus, such as the diffuse light of a cloudy day. In my own experience animals that have been kept in the dark for some time will usually react to light of not more than a few candle-meters intensity, but the same individuals after lengthy exposure to ordinary daylight will often fail to respond to a beam of strong sunlight. Obviously the capacity of the animal to respond to light is more or less determined by its previous condition, its sensi-

tiveness diminishing with continual exposure to light and increasing when the light is excluded from it. But even under the most favorable circumstances the reactions to light as compared with those to other kinds of stimuli are relatively slight in amphioxus.

Although amphioxus shows much diversity as to the intensity of light to which it will react, in another respect its responses to this form of stimulus are very uniform. In all the tests I carried out, I never observed a reaction to a rapid *diminution* of light, and the reactions to light that did occur were always the result of a rapid *increase* of intensity. When an animal was resting quietly on its side in a shaded aquarium and a beam of sunlight was suddenly thrown upon it, it would usually respond by one or two vigorous locomotor leaps, after which it might come to rest even in the sunlight. If now the sunlight was suddenly cut off, no response followed. That this failure to respond was not due to exhaustion from over-exposure to light was easily shown by quickly throwing on the sunlight a second time, whereupon a reaction much like the first one usually followed immediately. In fact, a moderately rapid alternation of full light and shadow was generally followed for a number of times by reactions to the light and no reactions to the shadow till, after numerous trials, the animal ceased to respond at all. Amphioxus is therefore stimulated only by such rapid changes of light intensity as involve an *increase* in the illumination. This agrees fairly well with Nagel's statement ('**94**, p. 811; '**96**, p. 80) that sudden shadow calls forth from amphioxus either faint responses or none at all. In my experience the latter part of this statement is correct.

Having ascertained that amphioxus is sensitive to light, the next question that naturally arises is what portion of its body serves as the receptive organ for this stimulus. Numerous answers have already been given to this question. The conspicuous pigment spot at the anterior end of the nerve-tube discovered, according to J. Müller ('**39**, p. 198), by Retzius, was held by the former ('**44**, p. 95) and many other investigators to be a primitive eye. Hasse ('**76**, p. 287) believed that the light receptors were two lateral patches of integumentary cells, one on each side of the flattened anterior end of the animal. Nüsslin ('**77**, p. 25) was of opinion that the extreme anterior portion of the dorsal fin was the part sensitive to light. Krause ('**88**, p. 136), who discovered in the substance of the nerve-tube a pigment that he believed resembled visual purple, was thereby led to assume that this tube was the receptive organ for light. Nagel ('**94**[a], p. 811) claimed that the whole outer skin was receptive to light. Hesse ('**98**[a], '**98**[b]) maintained that the numerous small pigment spots of the nerve-tube were each a

single eye comparable to the eye of a planarian ; and to these Joseph (:04) added certain large cells in the anterior part of the tube which, from their structure, he believed also to be light-receptors.

To ascertain what part of the body of amphioxus is sensitive to light, I had planned to use local stimulation, and with this in view I arranged an acetylin light with a condensing lens and a pinhole diaphragm, so that I could have at command a small beam of strong light with which to test locally the various parts of the animal's body. Unfortunately the strongest artificial light that I could get was insufficient to call forth an invariable reaction, and I was at last driven to use concentrated sunlight for this purpose. This was obtained by mounting a mirror in an open space adjacent to the laboratory, and so directing it that a horizontal beam of sunlight was thrown through a window into the laboratory. This beam of light was screened of its heat by being made to pass through seven centimeters of water contained in a glass vessel with flat sides, and it was concentrated by a large lens whose principal focus was about twenty-five centimeters. A few centimeters nearer the lens than its principal focus and in the cone of concentrating light, an iron diaphragm with a pinhole was placed that intercepted all the light except that which passed through the pinhole. In this way a well-circumscribed minute beam of intense light was obtained, and by means of this beam the body of the amphioxus was explored while it rested in a glass dish of sea water with flat sides. It was found by experiment that the dish containing the amphioxus could be moved about with considerable freedom without disturbing the animal. In this way the beam of light was brought to bear on any desired part of the animal's body.

My first experiments were directed toward ascertaining the value of the so-called eye-spot at the anterior end of the nerve-tube as a receptive organ for light. Experiments had already been made on this organ by Nagel ('94[a], p. 811 ; '96, pp. 40, 80), who recorded that after the animal's anterior end, including the eye-spot, had been cut off, the lancelet was found to be as sensitive to light as ever, a condition confirmed by Hesse ('98[b], p. 461). I repeated this experiment on six lancelets. All were first tested with light and found to respond when suddenly illuminated. The anterior tip of the body with the eye-spot was then cut off, and after an hour all were tested again. I was unable to distinguish in this second test that the lancelets were any less sensitive to light than before the removal of the eye-spot, and my results thus confirm those of Nagel and Hesse.

Although these results demonstrate conclusively that the so-called eye-spot is not essential to the light reactions of amphioxus, they do

not show that this spot may not be a light-receptive organ. To test this possibility I attempted by means of the minute beam of light already described to illuminate the spot exclusively, and to see if a reaction resulted. This was by no means easily done, for the spot is so small that its position in the living animal cannot well be observed directly, but must be surmised. Furthermore, when the light enters the substance of the animal, it becomes much scattered, and hence may reach other parts than those it is intended to illuminate. Nevertheless, it was possible on a number of animals to throw intense light on the eye-spot without getting a response, though, when the light was moved to a position somewhat posterior to the spot in question, a vigorous response followed. I therefore conclude that not only is the so-called eye-spot of amphioxus unessential to its light reactions, but that this organ is in no sense a light-receptor. These physiological results, then, support the view long ago advanced by Stieda ('73, p. 51) on the basis of anatomical evidence, that this spot is not a visual organ. For this reason I shall in future call it simply the anterior pigment spot, though its nervous nature seems well established by the recent work of Edinger (:06). In a similar way I tried to get reactions from lancelets by directing the beam of light on the flattened sides of their anterior ends, where, according to Hasse ('76), light-receptive organs were supposed to be located. In no instance did I get a reaction, and I therefore agree with Nüsslin ('77, p. 12) and with Kohl ('90, p. 183) in denying the existence of light-receiving organs in this region.

Lancelets from which the anterior end of the dorsal fin had been removed were as sensitive to light as before the removal, nor did normal lancelets react to the small beam of light when it was thrown on this part of the fin. I therefore believe that Nüsslin ('77, p. 25) was in error when he declared that the anterior end of the dorsal fin was the portion of the animal that was sensitive to light.

The part of the body of amphioxus that can be stimulated by light extends from a point a little behind the anterior end posteriorly to the tip of the tail. A beam of concentrated sunlight thrown across the body in any region between these two points always elicits some response. Krause ('97, p. 514) states that the anterior end somewhat distal [3] to the anterior pigment spot is most sensitive to light, and that the tail end is not sensitive at all. My results, as already stated, are almost precisely the reverse of these. I have found the anterior end, both in front of the anterior pigment spot and at least immediately posterior to it, insensitive to light, and the tail end extremely sensi-

[3] By *distal* Krause means, judging from the context, *posterior*.

tive. As Krause in his first description of the animal ('88, pp. 132 135) stated that it rests with its *tail* out of the sand, and in his later account ('97, p. 513) that the *head* usually projects, a fact well established since the time of J. Müller ('41, p. 399), is it not possible that in his study of the light reactions of this somewhat ambiguous form Krause has fallen into the not unnatural error of confusing the ends?

The extent of the region that is sensitive to light in amphioxus very nearly coincides with that of the nerve tube, and evidence obtained by local stimulation points to this structure as the part of the animal stimulated by light. Krause ('88, p. 132; '97, p. 513) has advanced the opinion that the bluish coloring matter that appears in the walls of the tube when this structure is treated with alkali is similar to the visual purple of the retina, and is in this way connected with the light receptive function of the tube. On treatment with alkali this coloring matter, according to Krause, becomes visible around the pigment spots in the tube, and among these are included the anterior pigment spot as well as the series of smaller spots that extend through almost the whole length of the tube; but it has just been stated that by local stimulation the anterior pigment spot can be shown to be insensitive to light, and since this coloring matter is as characteristic of that spot as of the other spots in the tube, I do not believe that the blue substance described by Krause has any essential connection with the light-receptive apparatus. As Hesse ('98[c], p. 556) has pointed out, Krause's belief that the blue is analogous to visual purple is unsupported by any good evidence, for this material shows no such relation to light as is characteristic of visual purple. It therefore seems to me that Krause's view is untenable.

Since amphioxus shows no response when strong light is thrown on the anterior end of its nerve-tube in front of the third or fourth segment, a region in which occur certain large cells supposed by Joseph (: 04, p. 21) to be sensitive to light, I conclude that these cells are not open to that kind of stimulation and that the light-receptive organs must lie posterior to this region.

Although it is impossible, for reasons already given, to illuminate amphioxus locally with great precision, the exact portion of the animal that is stimulated by light can be determined with fair accuracy. This portion corresponds to the region in which the nerve-tube contains the small eye-cups described by Hesse. This correspondence is so precise that it seems very probable that these organs are the true photoreceptors. It must not be forgotten, however, that, in all regions where light has proved stimulating, this agent in its passage into the more or

less transparent animal first penetrates the skin, and it is not impossible that the receptive organs for light really lie in this layer, as maintained by Nagel ('94[a], p. 811) and Jelgersma (:06, p. 390). This opinion is strengthened by what recently has been made out concerning the sensitiveness to light of the skin of certain reptiles, amphibians, and fishes, particularly ammocoetes (Parker, :03[c], :05[b]).

Since I was unable to devise an experiment whereby the nerve-tube in amphioxus could be illuminated without having the light pass through the skin, I cannot be absolutely sure where the light-receiving organs lie, but there is a certain amount of indirect evidence on this question, all of which points in one direction. As has already been shown, the skin on the anterior end of the animal is not sensitive to light, this form of sensitiveness beginning posteriorly at no special region so far as the skin is concerned, but exactly where the eye-cups first occur in the nerve-tube. This evidence, so far as it goes, favors Hesse's view that these eye-cups are the true light-receptive organs. Another piece of evidence has to do with the exact distribution of the animal's photoreceptiveness and that of the eye-cups. If different regions on the length of a lancelet are tested for their sensitiveness to light, they will be found to vary considerably. The most sensitive region is that which extends from a point several segments behind the anterior tip of the nerve-tube posteriorly over about one quarter of the length of the animal; the region next in sensitiveness is the most posterior quarter of the animal; and the least sensitive part of the whole region which is at all sensitive is approximately the middle half. In a series of trials in which was determined the relative intensity of the minimum amount of light necessary to stimulate in these three regions, it appeared that, if the minimum intensity for the anterior portion, the most sensitive part, is called 1, that for the posterior part was 1.5, and for the middle part 25.0, while an intensity of 0.5 was not stimulating to any part of the animal. If, now, the distribution of the eye-cups described by Hesse be taken into account, a striking correspondence to the sensitiveness to light will be found. In Branchiostoma caribbaeum the most anterior eye-cups occur in the third segment, and the remaining cups form a more or less segmentally arranged series reaching to the last segment of the body, which is practically the tip of the tail. In this series, so far as numbers are concerned, three general regions can be distinguished. The first region, the one in which the cups are most numerous, extends from about the fourth segment to about the twentieth; the region second in abundance covers about the last twelve segments of the body; and the third region, or the one in which they are fewest, is the middle portion of the body between the two regions

just defined. Hesse ('98[b], p. 457) states that in Branchiostoma lanceolatum the eye-cups are most abundant anteriorly and diminish in numbers posteriorly, till in the tail there may be not more than one cup to a segment. But this description, as Boeke (:02, p. 352) and Joseph (:04, p. 18) have noted, is somewhat defective. In five specimens of B. lanceolatum from Naples that I have examined, the distribution was essentially like that in B. caribbaeum, in that, in addition to the considerably increased number of cups anteriorly, there was also an increase in the number in the tail region. This confirms Joseph's statement (:04, p. 18) for this species and agrees with the discovery of Boeke (:02, p. 352), that in young pelagic individuals of B. lanceolatum there are to be seen *two* groups of eye-cups, one anterior and the other posterior, corresponding to the two concentrations mentioned. These two groups presumably unite later to form one series. The general plans of distribution of the cups in the two species, then, undoubtedly agree, and, since these plans of distribution correspond to the different degrees of sensitiveness to light for the different parts of the body in B. caribbaeum, I believe that the eye-cups described by Hesse, and not the skin, are the light-receptive organs.

In Branchiostoma caribbaeum, as in B. lanceolatum according to Hesse ('98[b], p. 458) and Boeke (:02, p. 351), the ventral eye-cups, as well as those of the right side, point in the main ventrally, while those of the left side point mostly dorsally. Hesse states further that in B. lanceolatum the cups of the two sides tend toward the right, and he suspected that this might be correlated with a possible habit of resting on a particular side. But in testing this hypothesis Hesse ('98[b], p. 459) found that the animals rested about as frequently on one side as on the other, and he therefore abandoned it. In B. caribbaeum I could not see that the cups were directed more toward the right than toward the left, but it was apparent that the majority pointed ventrally. This position seemed to me entirely consistent with the habits of this species, for it naturally lies in the sand with the ventral side obliquely *uppermost*, the majority of eyes being thus directed toward the most probable source for light. However, individuals that were in a glass dish without sand were, so far as I could see, equally sensitive to light falling on them in *any* direction.

If the light-receptive organs in amphioxus are the eye-cups of the nerve-tube, any part of the animal containing these organs might be expected to retain its sensitiveness to light. Nagel ('94[a], p. 811; '96, p. 79), after cutting these animals in two transversely, found that both halves still reacted promptly to light, but less energetically than the whole animal did. Krause ('97, p. 514) declared that after halving

amphioxus the posterior part is much less reactive to light than the anterior, and Hesse ('98[b], p. 462), who repeated these experiments, could get only a trembling response to light from the anterior half and no response at all from the posterior one. My own results agree exactly with those of Hesse. I tested six fresh animals with strong sunlight, and, having found them sensitive to it, I cut each one transversely in two. After an hour, and again after two hours, I tested them with strong sunlight: the anterier halves always trembled markedly, but I could perceive no reaction at all to light from the posterior halves. When, however, I touched the posterior halves with very dilute nitric acid in sea water, they sprang and wriggled forward through the water most energetically, showing that they were still capable of active response. I am therefore convinced that cutting the animal in two has a profound effect upon its powers of reaction to light, greatly diminishing this capacity in the anterior half and practically nullifying it in the posterior half.

Although amphioxus reacts to light thrown upon almost any part of its body except the anterior end, its reactions are characteristically different in accordance with the region stimulated. When light is applied to the sensitive anterior fourth of the body, amphioxus almost invariably gives a vigorous backward spring, often accompanied with backward swimming. If light is applied to the less sensitive middle portion of the body, there is usually a slight backward spring, but sometimes the animal simply curls the body slightly. If the light is applied to the most posterior fourth, the animal almost invariably springs forward. In extreme cases, at least, the resulting movement is the most effective one for removing the animal from the source of stimulation. This is still more clearly seen when a beam of strong light parallel with the longitudinal axis of the amphioxus is directed against its anterior or its posterior end. In the former case the animal darts backward, and in the latter forward; in each instance it moves away from the source of light. For animals generally backward swimming is unusual, since the majority of negatively phototropic animals when illuminated from in front first orient by turning the anterior end away from the light before they begin active locomotion, whereas in amphioxus the locomotion is executed without the initial step of orientation. The case is parallel to that of a positively phototropic pycnogonid described by Cole (:01, p. 201); this animal moves toward the source of light either with the anterior or the posterior end first. In the pycnogonid, however, the two kinds of movement are associated with somewhat different types of locomotion, for the animal *swims backward* toward the light or *creeps forward* toward it, whereas in amphioxus the reaction in both cases is simply swimming.

As a result of such a system of reactions, Branchiostoma caribbaeum falls under the head of negatively phototropic animals, and this is also the case with B. lanceolatum, which, according to W. Müller ('74, p. 7) and others, avoids light as far as possible when in captivity, and with Asymmetron lucayanum, whose habit, according to Andrews ('93, p. 214), is to collect on the side of the dish away from the light. Evidence of the same kind is also at hand for B. caribbaeum. If, into the middle of a large square glass vessel so placed that the sunlight falls obliquely into it through one side, living lancelets are dropped one by one, they fall to the bottom as a rule without response, whereupon they often begin swimming, and in practically every trial come to rest near the side of the glass away from the sun.

If a large glass aquarium is arranged so that one side and the halves of the two ends adjacent to it, as well as the corresponding portion of the top, are covered with light-proof paper and a number of amphioxus are allowed to swim freely about in it, they will be found during the day resting almost exclusively on the bottom of the darkened part, whereas during the night they will be found about equally distributed over the bottom.

Since amphioxus swims away from a source of light, it is negatively phototropic (Parker, '06, p. 61), and, since it is active in the light and comes to rest in darkened situations, it is photokinetic (photodynamic).

Light acts on amphioxus in a distinctly local way, and not as it does on animals, like most vertebrates, which possess eyes capable of forming images. This power enables a vertebrate to discriminate at a distance areas of light from areas of shade in a general field. If an amphioxus lying quietly in deep shade is stimulated to locomotion by a minute beam of strong light, it will dart off in almost any direction irrespective of the shadows and lights about it. Should it by accident come into the sunlight, it usually continues to swim; should it come into shade, it usually comes to rest. The light about amphioxus has little or no influence on the animal except when it falls with full intensity on the animal's body. This is dependent upon the fact that amphioxus is not very sensitive to light, and therefore reflected light of low intensity does not stimulate it, and, further, that the light-receptive organs of the animal have no adequate means for the formation of images.

Under ordinary conditions amphioxus is buried in the sand, excepting for one end. Which end this is has been a matter of some dispute. Yarrell ('36, p. 468) stated that the specimen from which he took his description was found by Mr. Cough with its tail sticking out from under a stone; and Steiner ('86, p. 497) declared that the animal

usually rests with its tail out of the sand, a statement repeated by Krause ('88, pp. 132, 135). Subsequently and without explanation both Steiner ('88, p. 41) and Krause ('97, p. 513) abandoned this opinion for the opposite one. That the animals ordinarily rest with the anterior end out of the sand was the opinion of J. Müller ('41, p. 399; '44, p. 84), Nüsslin ('77, p. 18), Rohon ('82, p. 37), Willey ('94, p. 9), Nagel ('96, p. 79), and others, and any one who carefully inspects a number of lancelets at rest will soon be convinced that this is the normal position. Although the extruded anterior end is the portion of the animal least sensitive to light, lancelets in their resting positions in ordinary sand will respond quickly enough to this stimulus. Thus in a large dish of coral sand, over which there were a few inches of sea water, the anterior ends of twenty-three lancelets were counted in dim light. As a result of throwing on a beam of very strong light, most of the heads were quickly withdrawn under the sand, only two remaining visible. This reaction is doubtless dependent on the stimulation of the most anterior eye-cups, and as a rule the resting position of the animal is such that this naturally occurs.

The negative phototropism of amphioxus has led to the belief that during the day it remains buried in the sand, except perhaps for its anterior end, but that during the night it leaves the sand and leads a more active existence. W. Müller ('74, p. 7) states that Branchiostoma lanceolatum is nocturnal, and at twilight comes to the surface of the sandbank in which during the day it is buried. Rice ('80, p. 9) mentions that individuals of this species which were seen swimming at night in the Naples Aquarium were quiescent in the daytime, and Rohon ('82, p. 36) and Krause ('97, p. 513) also speak of this species as having nocturnal habits. B. caribbaeum showed no evidence of such habits. All inspections of the aquaria that I made after nightfall, and with caution as far as light was concerned, demonstrated that the lancelets remained in the same position in the dark as in the light. Further, several glass vessels containing coral sand and known numbers of lancelets that were sunk over night to the natural level of the sand in the bed of the inlet, contained, when taken up the next day, the same numbers of animals, thus indicating that the lancelets had remained buried and had not come out on the surface of the sand, where the current would surely have swept them away, even supposing that they had not started swimming. Although this experiment was tried only a few times, the results always led to the same conclusion, and it therefore seems probable that at least B. caribbaeum is essentially a burrowing animal, and that it leaves its native sand only when forced to by the accidental action of currents, etc.

3. Heat.

The reactions of amphioxus to heat have been scarcely more than touched upon by the numerous investigators who have studied the reactions and habits of this animal. As has already been pointed out, the opinion of Rohon ('82, p. 38) and of Kohl ('90, p. 185), that the light reactions of amphioxus are really reactions to radiant heat, is erroneous; moreover it is not to be expected that animals like amphioxus, which live always under some depth of water, would have any special organs for the reception of radiant heat, since such heat penetrates water only a centimeter or two and hence would almost never reach these forms. The kind of heat that is a factor in the environment of amphioxus is the molecular vibration such as we recognize in the temperature of water, and this certainly has a distinctly circumscribing influence on the lancelets.

In testing the effect of heat on amphioxus, the temperature of the water in which they were living in the Flatts Inlet, 31° C. (July, 1905), was taken as the normal, and two series of experiments were conducted, one at temperatures above this and another at temperatures below it.

When lancelets were transferred from sea water at 31° C. to sea water at 35° C., they responded by darting about several times and then sinking quietly in the characteristic way to the bottom of the dish. Their subsequent reactions were essentially normal.

When transferred to sea water at 37° C., they made several quick darts, and finally fell quietly to the bottom, where they rested. When under these circumstances dilute acid was applied to them, they were found still to be actively responsive.

When transferred to water at 40° C., they made one or two sudden plunges, after which they dropped to the bottom, while their semi-transparent substance gradually whitened. When touched with dilute acid, the animals quivered slightly, but did not react otherwise. In a short time they were dead.

At 42° C. the animals darted once or twice, whitened quickly, and dropped to the bottom dead. Bert ('69, p. 21) states that water at 41° C. kills amphioxus in two minutes.

At 45° C. no locomotor response at all was given, and the animals began to whiten at once; they were apparently dead before they reached the bottom of the dish.

It is plain from these records that heat has at least two influences on amphioxus. It stimulates them to momentarily vigorous locomotion, and it also brings about death by the coagulation (whitening) of certain materials in their living substance. The coagulation begins

apparently at about 40° C., and may be so rapid at 45° C. as to prevent the characteristic locomotor reaction which occurs at lower temperatures.

Having ascertained something of the general effect of heat on amphioxus, I next endeavored to determine what parts of its body were sensitive to this stimulus. To this end I used a temperature 39° C., a little lower than that which caused coagulation. I attempted to apply this temperature locally by touching the animal in the region to be tested with a sharply bent glass tube kept at the required temperature by a rapid flow of hot water through it. The bent tube thus heated was applied successively, but at considerable intervals, to the anterior end, middle, and tail of several animals, and their reactions recorded. As a check on this method the bent tube filled with water at 31° C. was also applied to the animals, with the outcome that the mechanical stimulation was found to be so considerable that the results dependent upon temperature could not be rightly judged, and the method was therefore necessarily abandoned.

I next tried running a gentle stream of warm sea water on different parts of the lancelet's body while it was resting in a dish of sea water at 31° C., and I checked this method by using the same strength of stream, but at the normal temperature. This procedure proved much more satisfactory than the use of the bent tube, for the current of water at the normal temperature seldom, if ever, gave rise to a response, while that at 39° C. very generally did.

When the heated current was applied to the anterior end of a lancelet, the animal very usually swam immediately backward a short distance. When it was applied to the tail, the animal often moved forward. When it was applied to the middle of the body, the reaction never was locomotor, but only a slight bending or jerking of the body, and even this was apparent in only about one out of every ten trials.

The reactions of amphioxus on being immersed in warm water or touched by a current of warm water follow so quickly on the application of the stimulus that I am convinced that stimulation takes place on the surface of the animal, for there was scarcely time for the heat to reach by conduction any relatively deep-lying part. I therefore conclude that heat is a sensory stimulus for amphioxus, and that it is very probably effective for the whole outer surface of the animal, the head being most sensitive to it, the tail less, and the middle portion of the body least.

In a second series of tests, water cooler than 31° C. was used with which to stimulate the amphioxus. When animals were transferred from water at 31° C. to water at 25° C., they swam about with

more energy than at the normal temperature. Finally they dropped quietly to the bottom.

At 20° C. they swam very energetically and near the top of the water, but finally dropped to the bottom; subsequently, on being touched with a rod, they swam, but not so energetically as at the normal temperature.

At 15° C. they swam vigorously, but soon dropped to the bottom.

At 10° C. they passed into the water without swimming, dropped to the bottom, and remained quietly there.

At 5° C. they behaved as at 10° C. After remaining on the bottom at 5° C. for five minutes, they were removed to water of ordinary temperature, where their reactions seemed to be entirely normal.

Five active amphioxus were then dropped into water at 4° C., and after half an hour they were tested and all found to be dead. The temperature of the water at the end of half an hour had fallen to 2.5° C. This experiment was several times repeated, and always with the result that death followed exposure to extreme cold for half an hour or so.

Cold water from 25° C. to 15° C. is certainly stimulating to amphioxus. At 10° C. and lower no response is given, but death may intervene, particularly at lower temperatures, from unknown causes.

All attempts at local stimulation with cold water were entirely unsuccessful. Water at 15° C., when applied as a current to the anterior end, tail, or trunk, was without effect, though, as already mentioned, immersion in water at this temperature called forth vigorous swimming. A current of water at 2° C., when applied locally to the anterior end, tail, or trunk, gave rise, as might have been expected, to no reaction.

The reactions to cold water, when they occurred, were quite as quick as those to warm water, and must therefore have been the result of a very superficial stimulation; but whether this was a stimulation of the whole outer surface, or of a special part of it, or of some special region like the entrance to the mouth, I am unable to say.

The fact that amphioxus swims away from any source of considerable heat places it among negatively thermotropic animals. That it can be stimulated to active, non-directive swimming by both heat and cold shows it to be thermokinetic. That it should be stimulated by cold, but not influenced in a directive way by this stimulus as it is by heat, favors the view that it possesses, like some higher vertebrates, separate receptors for heat and for cold.

4. Mechanical Stimulation.

As has been pointed out already, the apparently great sensitiveness of amphioxus to light is really sensitiveness to mechanical stimulation, a form of sensitiveness long ago remarked by Merkel ('80, p. 7), who observed that a vigorous amphioxus would respond by very active locomotion to the lightest touch of the forceps.

To test the reactions of amphioxus to mechanical stimulation I first used a course pig-bristle mounted so that the rounded end could be brought into contact with any part of the animal's exterior. When the anterior end of an amphioxus resting in a shallow dish of sea water was touched even lightly with the bristle, the animal usually sprang backward, though occasionally forward. The backward spring was often accompanied by a somersault-like movement, whereby the animal became turned end for end. When the stimulus was applied to the posterior part of the body, the result was almost invariably a forward leap. This portion of the body, though sensitive, was not so much so as the anterior end. The middle of the body was much less sensitive than either of the ends, and when the tip of the bristle was applied to it, there was often no reaction. When, however, a reaction did occur, it was almost always a backward leap.

In general the reactions of amphioxus to mechanical stimulation resemble in essential respects their reactions to light, showing that the anterior end of the animal is most sensitive to such stimuli, the posterior end less so, and the middle of the body least, and that backward locomotion usually results from stimuli applied at the anterior end or the middle, and forward locomotion from stimuli at the posterior end.

By means of local stimulation the sensitiveness of different portions of the body could be roughly determined. At the anterior end, though the rostrum can be stimulated, the most sensitive parts are the oral hood and the buccal cirri. When any of these parts is touched, backward locomotion almost invariably follows. If the hood, but especially the cirri, are touched only very lightly, they close and open with a sudden movement not unlike winking. In resting animals this is often carried out in what seems to be a spontaneous manner, but close inspection shows that it is dependent upon the accumulation on the cirri of debris from the current of water usually passing in at the anterior end. When the cirri become fairly covered with minute particles of coral sand, etc., this winking movement loosens these particles, and at the same time vigorously expels the water from just within the anterior opening of the animal, and thus removes the ac-

cumulated debris. This reaction is doubtless dependent upon the mechanical stimulation caused by the particles of sand, etc., on the cirri, for, as already stated, the momentary contact of the end of the bristle with the cirri will call it forth.

The great sensitiveness of the anterior end of amphioxus, which has already been noticed by Krause ('88, p. 146), is resident chiefly in the outer surface of the oral hood. This part of the animal is easily stimulated by contact with any moving body and is the region especially concerned with the reception of stimuli when, through the movements of a few individuals, a whole assembly is set in violent commotion. It is also probable that this part is especially stimulated when an amphioxus, almost buried in sand, is made to draw back under the sand by directing a fine stream of water on the exposed anterior end.

In the middle-trunk region the firm dorsal and lateral walls, and even the delicate ventral one, are relatively insensitive to mechanical stimulation.

The whole of the caudal region is more sensitive to mechanical stimuli than the trunk region, but less so than the anterior end. The surface about the atrial pore is especially sensitive to touch, and a stimulation of this region not only results often in forward locomotion, but also in a wave of contraction that passes anteriorly from the atrial opening over perhaps half the length of the thin ventral atrial wall.

As amphioxus is so easily stimulated by gross mechanical disturbances, it is not surprising to find that it will respond to such delicate mechanical stimuli as sound waves. If a glass vessel that contains resting amphioxus partly buried in the sand is gently tapped on the side, the animals, as Rice ('80, p. 8) long ago observed, usually withdraw temporarily below the sand, or at least move their cirri in a way that resembles winking. That this is not due to the vibration of particles of sand against their bodies is seen from the fact that at least the reaction of the cirri can be called forth from animals that are resting on a bed of cotton wool in a glass vessel of sea water when the walls of the vessel are tapped. Another common form of response to sound vibrations, often seen under the conditions just mentioned, is a wave-like contraction of the atrial membrane. This membrane in fact is so placed that it may be especially open to stimulation by sound waves, for it is suspended between the atrial cavity and the outer space, both of which are filled with sea water.

It is very probable that all these reactions to sound depend upon the stimulation of some part of the tactile mechanism, for in the first place amphioxus has no special organ that can serve it as an ear (Stieda,

'73, p. 52), and secondly, many sound vibrations can be sensed through our tactile organs as well as our ears.

That mechanical stimulation serves as a basis for thigmotropic, geotropic, and even rheotropic reactions cannot be doubted, though Lyon (:05) has shown that rheotropism in certain fishes depends more upon sight than upon touch. All three kinds of reactions are shown by amphioxus.

The thigmotropism of amphioxus is evident from the following experiments. Ten amphioxus were liberated in a flat-bottomed glass aquarium containing a depth of 10 centimeters of sea water and five centimeters of coral sand. After half an hour all the animals had buried themselves in the sand, and after an hour and a half seven of them had come to rest with their anterior ends a little above the level of the sand, their usual position (p. 426). That these reactions were not the result of the light that fell into the dish from above is seen from the fact that similar reactions were obtained from animals that were liberated in a covered glass dish of sea water containing a layer of sand between one and two centimeters thick and illuminated by a mirror from below only. Under these circumstances the amphioxus came to rest in the sand, but in such positions that in many cases their bodies were exposed to light through the glass bottom of the dish, though their anterior ends projected into the darkness above the sand. Thus it is evident that they did not enter the sand to escape the light. Moreover, amphioxus will rest quietly, much as when it is in sand, provided all but its anterior end is covered with small fragments of glass. Through this covering the light may pass to the animal, and apparently this does not disturb it, for its quiescence seems to depend merely upon the contact of its body with the particles of glass. I therefore believe that amphioxus is thigmotropic.

The movements by which amphioxus buries itself are not without interest. As a rule the animal dropped passively through the sea water to the sand below. When it came in contact with the sand, it sometimes gave a sudden spring and disappeared below the surface. More frequently, however, it straightened out upon the sand, as noted by Müller ('44, p. 84) and by Willey ('94, p. 10), and later, particularly if it was moved by a current, it would arch and disappear below the surface, as described by Rice ('80, p. 8). Its disappearance into the sand was so quickly accomplished that it was impossible for me to ascertain by direct observation whether the animal entered the sand with the anterior end first or the tail first. Steiner ('86, p. 497) maintains that the anterior end of the animal enters the sand first, and that it may continue to burrow through the sand till this end emerges. He

further asserts that the animals are incapable of burrowing with the tail first. Müller (41, p. 399), however, in his description of the animal's habits implies that it enters the sand tail first, and often burrows only far enough to cover the main portion of the trunk, leaving the anterior end exposed. I attempted to ascertain the truth of the matter by carefully uncovering animals that had buried themselves, thus determining by direct inspection which end had probably entered the sand first. I also noted in instances where the animal had failed to cover itself completely which end was left exposed. These instances were more conclusive than those of completely covered animals, for in these cases there was no chance for an unobserved reversal of ends as might occur where the animals were for a short time out of sight. In the great majority of these cases the animals had evidently entered the sand tail first, though there were some instances, especially among the imperfectly covered ones, in which it was clear that they had entered with the anterior end first. Other evidence on this question was derived from animals on which a slight operation had been performed. Amphioxus from which a part of the tail had been removed entered the sand only after many trials, whereas others whose rostrum had been cut off but whose tail was intact seemed to have no difficulty in making their way into the sand. These observations are in agreement with what was noticed in animals that had partly or completely buried themselves, and I am therefore convinced, notwithstanding Steiner's statement to the contrary, that amphioxus usually enters the sand tail foremost.

In one respect the amphioxus buried in the sand were very different from those lying freely on the surface. The free individuals were usually very straight, as though held in form by the stiffness of the notochord. The buried individuals, on the other hand, had when in the sand a very tortuous outline, as though they had crowded their way in between the coarse pieces of shell and coral. Such individuals immediately became straight on being released from the sand.

Rheotropism, though present, is not a prominent feature in the reactions of amphioxus. In the inlet at the small landing pier in front of Hotel Frascati large schools of small fish could be seen definitely oriented in reference to the swift current. These schools maintained a more or less constant position by swimming against the current about as rapidly as the current would have carried them in the opposite direction. When living amphioxus were dropped into these schools, they drifted among the small fish on the way to the bottom without as a rule the least locomotor movement, and, when they did move, they never showed any tendency to orient to the current. Moreover, when

they were placed in a floating aquarium the sides of which were of netting so as to permit a strong current of sea water to pass through it, they either drifted to the far end of the aquarium or swam irregularly about and without reference to the current, though a few small fish that were caught and put into the aquarium swam against the current with precision.

These observations are in agreement with what Lyon (:**05**) found as to the rheotropism of certain fishes, namely, that in large general currents their orientation is dependent not upon the direct stimulus of the current, but upon the possession of a visual organ capable of forming an image whereby they could fix their position in reference to motionless objects on the banks and in the bed of the stream. Since amphioxus does not possess visual organs of such a character, orientation under these circumstances is not to be expected.

If, however, an amphioxus is put into a large cylindrical vessel filled with sea water and the water is made to whirl in it, the animal is quickly stimulated to swimming and swims vigorously against the current. After a short period of active swimming, in which the animal will often progress more rapidly than the current moves in the opposite direction, it will drop to the bottom as though exhausted and be carried round and round by the water. It was evident from the movements of the animal that the stimuli to its locomotion were the momentary contacts with the inner sides of the vessel next which it was often swept and in all probability the varying rates of those parts of the current that touched the sides of the animal. To such an irregular current amphioxus undoubtedly reacts, *i. e.*, under these circumstances it is rheotropic.

Amphioxus can also be shown to be slightly geotropic. This feature does not appear in its swimming, for though Steiner ('**86**, p. 498; '**88**, p. 43) affirms that the whole animal, or even a quarter of it, will swim with full equilibrium, and is so quoted by Ayers ('**92**, p. 318) and by Sherrington ('**99**, p. 1276), my own observations agree with the statements of Rice ('**80**, p. 8) and of Willey ('**94**, p. 10), that in swimming amphioxus may move with any side uppermost and may continually change that side. This change of attitude during locomotion was so constant a phenomenon among the many amphioxus that I watched that there is not the least question in my mind that this animal during locomotion assumes no uniform position in reference to gravity.

In its resting state, however, amphioxus shows some slight response to gravity. As it lies on the sand it may rest for considerable periods of time with any side uppermost, but after it has burrowed and come to rest near the surface of the sand, it usually lies, as Rice ('**80**, p. 8)

and Hesse ('98b, p. 459) have noted, with the ventral side uppermost and always with the anterior end higher than the posterior. This relation of the two ends might be supposed to be due to the need of having the anterior end in clear water, and therefore to be a reaction to the water and sand in the surroundings and not directly to gravity, but that this assumption is false is seen from the following experiments. If several amphioxus are placed in a closed box made of coarse wire gauze and filled with sand and the whole immersed in sea water, in a few hours they will be found at the top of the sand with their anterior ends projecting into the sea water. If now the box is cautiously inverted, some of the animals will keep their original positions, and thus their anterior ends will project from the under side of the box into the adjacent sea water; but they will remain here only a short time, for sooner or later they will make their way upward through the sand to the top. In a similar way if, after they have come to rest at the top, the box is rotated through a quadrant so that their anterior ends project sidewise into the sea water, they will again desert this position and move to the top. Further, if in a funnel whose stem has been broken off short an amphioxus is buried in sand in such a way that its anterior end projects downward out of the small end of the funnel into the sea water, it will leave this lower end and make its way upward through the sand to the top, even if, in doing so, it emerges on sand above the level of the water. It is therefore evident that amphioxus will come to rest in the sand only when its anterior end is above its posterior one, and, from the conditions under which this occurs, such responses seem to be strictly geotropic.

5. Chemical Stimulation.

The chemical sense of amphioxus, as remarked by Nagel ('94b, p. 192), is not unlike that of a worm in that its seat is the whole outer surface of the animal and not simply the region around the mouth. This sense is doubtless serviceable chiefly as a means toward escape from unfavorable chemical surroundings and probably has little or nothing to do with the direct feeding habits of the animal. As is well known, amphioxus does not seek its food, but takes what is brought to it in water currents, selecting from this supply only in the crudest fashion, if in fact it can be said to select at all. Nagel ('94b, p. 58) has shown that the outer surface of amphioxus is sensitive to chloroform, etc., and declares that, notwithstanding the presence of the so-called olfactory pit near the anterior end, one part of the animal's body is about as sensitive to chemical stimulation as another, though the tail may possibly be more sensitive than any other portion.

In testing amphioxus for chemical responses I used solutions of sour, sweet, bitter, and alkaline substances, as well as solutions of certain oils and other materials. All these solutions were made up in sea water, and, where the strength is expressed as parts of a molecular solution, sea water was used as a basis for this mixture instead of distilled water.

For a sour substance I used nitric acid. If a pipette full of sea water is discharged gently on the side of a resting amphioxus, there is usually no reaction. On animals thus previously tested a few drops of a $\frac{m}{10}$ solution of nitric acid were discharged successively on the anterior end, on the middle, and on the posterior end. In all these trials vigorous locomotion was induced; backward when the region of application was the anterior end or the middle, and forward when it was the posterior end. When a $\frac{m}{100}$ solution was applied to the anterior end or to the tail, the characteristic reactions were obtained, but there was usually no reaction when this solution was applied to the middle of the animal. A $\frac{m}{500}$ solution called forth no reaction when applied to the middle or the tail, but only when applied to the anterior end. A $\frac{m}{1000}$ solution called forth no reactions at all. Hence to solutions of nitric acid the anterior end is most sensitive, the tail next, and the middle least.

A more detailed study of the anterior end showed the following conditions. In an animal that in its normal state responded when this end was stimulated by a $\frac{m}{500}$ solution of nitric acid, the removal of the rostrum and the olfactory pit made no observable difference in its responses, thus confirming Nagel's statement ('94b, p. 192) that the olfactory pit is not essential to the special chemical sensitiveness of the anterior end. This pit, which was first described by Kölliker ('43) and was believed by him to be olfactory in function, was found in living animals to be lined with ciliated epithelium, by the movement of which particles of carmine were carried into it from its *posterior* edge and discharged from it *anteriorly*. Cutting off also the buccal cirri left the animal still receptive to a $\frac{m}{500}$ solution. When, however, enough of the anterior end was removed to take away the velar tentacles, what remained could be stimulated only by a $\frac{m}{100}$ or a stronger solution of nitric acid. The high degree of sensitiveness of the anterior end is therefore dependent upon parts not farther posterior than the velar tentacles. Since these tentacles and the buccal cirri are abundantly supplied with groups of sense cells (Willey, '94, p. 20), it is not impossible that the great sensitiveness of the anterior end is due to these groups of cells; but to this question I can give no conclusive answer.

To make an alkaline solution, one per cent of potassic hydrate was added to sea water, with the result that a somewhat milky precipitate was formed. The filtrate from this mixture had a strongly alkaline taste, but it did not call forth any response when it was applied either to the tail or to the middle of amphioxus. At the anterior end it caused the animal to dart backward vigorously.

For a bitter material picric acid was used. About a $\frac{M}{50}$ solution is very near saturation in sea water. To this solution, when applied to the tail, middle, and anterior end, amphioxus reacted with characteristic locomotion. All three regions were also stimulated by a $\frac{M}{250}$ solution, but locomotion usually did not result. At $\frac{M}{1250}$ occasional slight reactions were obtained, but only when the solution was applied to the anterior end, the tail and middle being apparently insensitive to this strength.

When a ten per cent solution of cane sugar in sea water was discharged freely over the anterior end, the middle, or the tail of amphioxus, no reaction of any kind was given.

No reactions were observed when the surface of the animal was bathed with sea water containing the following substances in solution: ether, chloroform, turpentine, oil of bergamot, and oil of rosemary. However, when any of these materials in a pure state was applied directly to the skin of amphioxus, a vigorous locomotor response was elicited, as Nagel ('94[b], p. 58) had previously found for chloroform and oil of rosemary.

A one per cent solution of alcohol in sea water called forth no response when applied to the anterior end, the middle, or the tail of amphioxus. A five per cent solution stimulated the anterior end and tail but not the middle, and a ten per cent solution stimulated all three parts.

Not only are many chemical solutions stimulating to amphioxus, but fresh water is likewise. When animals were dropped into sea water to which had been added one-fourth fresh water, the animals were observed to swim for a time more vigorously than in pure sea water. When the sea water was diluted by an equal volume or more of fresh water, the amphioxus swam most vigorously, and in very dilute sea water or in fresh water they quickly died, as already observed by Bert ('69, p. 21) and by Johnston ('05, p. 115). These various mixtures were also locally stimulating. The mixture of one-fourth fresh water and three-fourths sea water induced a slight backward movement when applied to the anterior end, but apparently stimulated no other part of the body. All mixtures containing more than one-fourth fresh water stimulated both the anterior end and the

tail, but not even pure fresh water stimulated the middle of the animal. When any of these stimulating mixtures were applied to the head, the animal swam backward; when they were applied to the tail, the locomotion was forward.

These experiments show that the surface of amphioxus is stimulated by solutions of nitric acid (sour), potassic hydrate (alkaline), picric acid (bitter), and alcohol, and by strong ether, chloroform, turpentine, etc. It is also stimulated by sea water diluted with fresh water, a mixture of which may prove fatal. Such stimuli were most effective at the anterior end of the animal, less so at the tail, and least of all at the middle, and the reactions were always such as to enable the animal to avoid the stimulus. So far as these tests go, amphioxus may be said to be uniformly negatively chemotropic.

6. Interrelation of Sensory Mechanisms in Amphioxus.

The distribution of sensitiveness of amphioxus to the stimuli discussed in the preceding sections follows a very simple plan. To light, heat, mechanical and chemical stimuli, the anterior portion of amphioxus is more sensitive than the tail, and the tail is more sensitive than the middle region of the trunk. A more accurate comparison of the distribution of sensitiveness has shown that a response to light cannot be elicited when the most anterior part of the body is illuminated, though this region is very easily stimulated by either heat, mechanical or chemical means. This fact and the agreement of the degrees of sensitiveness to light with the numbers of eye-cups in different parts of the nerve-tube have been given a reason for the conclusion that the light receptors in amphioxus are the eye-cups themselves and not the nerve terminals in the skin. Since the receptors for heat, mechanical and chemical stimuli, lie in the skin, they must be distinct from the photoreceptors. Further evidence of this separateness is, however, seen in results obtained by exhaustion. If the tail of an amphioxus is stimulated by concentrated sunlight ten or twelve times, the animal will reach a state in which it no longer responds to the illumination. While in this state it will react, however, with great certainty when its tail is stimulated by water as 37° C., by contact with a camel's-hair brush, or by a $\frac{m}{40}$ solution of nitric acid. Thus from the standpoint of exhaustion the receptors for light can be shown to be physiologically distinct from those for the other stimuli.

The extent to which separate receptors in the skin might be distinguished for the several effective stimuli cannot be judged by the distribution of sensitiveness for these stimuli, because, so far as I could make

out, this distribution was the same for all such stimuli. Evidence on this point was to be had, however, from the following experiments on exhaustion. After about twenty applications of a $\frac{m}{40}$ solution of nitric acid to the tail of an amphioxus, the animal usually ceased to respond to this stimulus. But on testing the same part of its body with water at 37° C. or with contact from a camel's-hair brush, it was found to be immediately responsive. In a similar way about thirty vigorous strokes of a camel's-hair brush were needed on the tail of an amphioxus before it ceased to react to this form of stimulation, whereupon it was found still to be sensitive to water at 37° C. and to a solution of nitric acid. Finally after an animal had ceased to react to water at 37 C. it was still sensitive to contact with the brush and to acid. Thus, notwithstanding the fact that the distribution of sensitiveness for these several stimuli is such as to leave the question as to separate receptors unsettled, exhaustion shows very conclusively that their operations are physiologically distinct (Parker, '07, p. 724),and as there is no evidence that they may not be represented by separate terminal organs in the skin, I believe that such organs are probably present. To what extent a further discrimination might be possible, as, for instance, the separation of terminal organs for cold and for heat, or for the different kinds of chemical stimuli, cannot be stated, for no experiments in this direction were undertaken.

To all the forms of effective stimuli that I employed, amphioxus responded in but one way, namely, with such movements as would remove it as directly as possible from the presence of the stimulus. When the stimulus was applied to the anterior end or to the middle trunk region, the animal moved backward, and when the application was to the tail, it moved forward. In not a single kind of stimulus did the animal move regularly toward the stimulus. This negative response, which seems to pervade the whole sensory activity of amphioxus, is the basis of its habit of retreat and characterizes much of what it does. Even feeding, which is so usually a positive operation with animals, is in amphioxus a relatively passive affair and unconnected with any seeking reactions. It therefore seems that the whole sensory system of amphioxus is employed as the initial mechanism in removing the animal from possible danger rather than as an apparatus for leading it successfully into new territory. This feature, as Steiner ('88, p. 42) has already remarked, is perhaps the most striking peculiarity of the sensory reactions of amphioxus.

The negative response of amphioxus to stimulation is of importance in considering the question of the direction in which it swims. Rice ('80, p. 8) declares that amphioxus always swims with its anterior end

foremost and that he never saw it move with its tail in advance. Steiner ('86, p. 497; '88, p. 41) also asserts that the animal moves with the anterior end foremost. The locomotion of amphioxus is a rapid, curiously irregular wriggle, often accompanied with somersault-like movements which make it impossible to be sure at any moment whether the animal is swimming backward or forward. The results of momentary stimulation, however, show very conclusively that amphioxus can swim both backward and forward, and that the direction of swimming at the beginning of any course is dependent upon the part of the animal's body that was stimulated. But how long amphioxus keeps to one form of movement I was unable to discover. The fact that it usually buries itself in the sand tail first (p. 433) leads me to believe that, though it can swim forward, as maintained by Rice and by Steiner, it usually swims backward.

Another feature of the reactions of amphioxus is their great energy, which is quickly followed by what seems to be complete collapse. For a few moments the animal swims with the utmost vigor, and then drops down quite motionless, as though it had become entirely exhausted (Rice, '80, p. 9). That this is not exhaustion is seen from the fact that a slight stimulus will usually cause a second round of activity; but after a few such efforts, the animal becomes unresponsive to further stimulation and is doubtless temporarily exhausted.

7. Central Nervous System and Sensory Mechanisms in Amphioxus.

To what extent the uninjured central nervous system of amphioxus is essential to its sensory reactions has already been briefly alluded to in the account of this animal's reactions to light (p. 424), but now that the other classes of stimuli have been described a more extended discussion of this subject may be undertaken. Steiner ('86, p. 498; '88, p. 43), who was apparently the first to investigate the functions of the central nervous system in amphioxus, states that after an animal had been cut into two, three, or even four parts, all the parts reacted to mechanical stimulation by swimming forward, and from these observations he concluded that the central nervous system of amphioxus is a metameric structure without sufficient differentiation to allow one to divide it into brain and spinal cord. Although his description of the reactions of the pieces of amphioxus might lead one to infer that these fragments reacted exactly as the whole animal did, it is plain from his further account that such fragments were less sensitive than when they made a part of the whole animal; for he goes on to remark that, when

the sensitiveness of the fragment becomes much lowered, it is only necessary to put the piece in very dilute picric acid to call forth the characteristic locomotion again. Johnston (:05, p. 124), however, states that even a small piece of the tail of amphioxus can swim well and behaves much as the whole animal does. Nagel ('94[a], p. 811; '96, p. 79) declares that both halves of an amphioxus react promptly to light, but less energetically than the whole animal does. But Danilewsky ('92) maintains that the halves react, at least to mechanical stimuli, very differently; the anterior half is quite sensitive to this form of stimulus, but the posterior half can be brought to react only with difficulty. Krause ('97, p. 514) declares that the anterior half reacts vigorously to light and the posterior half only slightly. Hesse ('98[b], p. 462), however, states that after division the anterior part only trembles on being illuminated and the posterior part gives no reaction whatever.

My own observations on B. caribbaeum lead me to believe that whether reactions will be given by both halves of this amphioxus or not depends quite as much upon the nature of the stimulus as upon any other factor. To light, as already stated, I have never been able to get any response from the posterior half, though the anterior half regularly trembled whenever strong light was thrown upon it. In these respects my results agree exactly with those of Hesse, and they were, moreover, so uniform and regular that I am led to suspect the accuracy of Krause's and of Nagel's statements, at least so far as they apply to the posterior half of amphioxus. After the nerve-tube is cut, this part seems no longer able to respond to light. That this is due to the small number of eye-cups in this region, as Hesse believed, is not true, for, as a matter of fact, these cups are almost as numerous in the tail region as in any other part of the animal. In my opinion the failure of the posterior half of amphioxus to react to light is not due to the lack of sensitiveness, but to the interruption of some centrally situated, reflex path. In the posterior half, apparently, the sensory neurones that are stimulated by light cannot transfer their impulses directly to the motor neurones of the same region, but only indirectly through the anterior part of the nerve-tube; hence when this is removed the reflex ceases. It is in this way, rather than through altered sensibility, that an explanation of this phenomenon will, I believe, be found.

To mechanical, and especially to chemical, stimuli I found both halves of amphioxus to be responsive, not, however, as Steiner describes, but rather as stated by Danilewsky, in that the anterior part was found to be quite sensitive and the posterior part slightly so. These observations suggest that the central tracts over which photic

impulses pass are separate from those which transmit sensory impulses from the integumentary terminals. Since they show, further, that the anterior half of the nerve-tube is different in function from the posterior half, they are opposed to Steiner's view of a metameric nervous system with equivalent segments, and favor the opinion advanced by Ayers ('90b, p. 223) and supported by Danilewsky ('92), that the anterior end of the nerve-tube of amphioxus is already a primitive brain and the posterior portion a spinal cord.

8. Sensory Mechanisms in Amphioxus and their Relations to Vertebrate Sense Organs.

The conditions presented by the sensory mechanisms in amphioxus give some clue to what was probably a step in the differentiation of the sense organs in primitive vertebrates. In these forms tactile organs doubtless covered the whole exterior, as they now do the body of amphioxus and that of the higher vertebrates, but these primitive ancestors, like amphioxus, probably possessed nothing by way of differentiations of these organs. Such differentiations are represented by the lateral-line organs and the ears, both of which occur in the cyclostomes and the higher vetebrates, but are wholly unrepresented in amphioxus, for the ear supposed by Peters ('77, p. 854) to have been seen in this animal is well known not to occur there. From the embryology of these organs it seems probable, as Ayers ('92) has pointed out, that specialized tactile organs gave rise to lateral-line organs, and that from certain of these lateral-line organs the ear was differentiated. This history, based upon morphological considerations, is parallel to what is known of the physiology of these parts, for the lateral-line organs are stimulated by material vibrations of low rate (Parker, :05a; :03a; :03b), possibly also effective as tactile stimuli, and the ear is stimulated by material vibrations of a higher rate, such as we recognize as sound. In my opinion the stimuli for these three sets of sense organs may often overlap and the three sets of organs constitute a genetic series, in which the tactile organs are the oldest members and the ear the newest. Although the primitive functions of these parts were doubtless (1) touch, (2) reception of slow vibrations, and (3) hearing, all these parts, but especially the ear, became involved more or less in the reflexes of equilibrium. This relation, however, I believe to have been entirely a secondary one, and not in any way to represent the original function of these organs as intimated by Lee ('98); hence I have avoided any such expression as equilibration sense. Amphioxus thus represents an ancestral vertebrate with tactile organs, but without lateral-line organs or ears, and

in it the equilibration reflex can be said scarcely to have developed as yet. In this respect it is like a young lobster before the statocyst has been formed (Prentiss, :01), and its powers of orientation to gravity, revealed in only a slight geotropism when at rest, are correspondingly small.

As the receptive organs for mechanical stimuli probably represent a primitive stage from which the lateral-line organs and the ears of the higher forms have developed, so the receptors for light doubtless give some idea of what served as a source for the lateral eyes of vertebrates. It has already been pointed out that the only organs that are known to be light receptors in amphioxus are the eye-cups. Hesse ('98b, p. 462), however, who was most instrumental in establishing this fact, does not regard these organs as in any way the homologues of the vertebrate eye, and in this opinion he is followed by Joseph (:04, p. 25). But I must confess that to me the evidence seems to point very definitely to the conclusion already drawn by Boveri (:04, p. 411) that the sensory cell of each eye-cup is homologous to a rod- or a cone-cell. In my opinion the eye-cups of amphioxus represent a diffuse sensory material from which an eye, like the lateral eye of the vertebrate, or even a series of eyes, as suggested by Locy ('97), could have developed, much as the ears of these animals have been differentiated from their lateral-line organs. The objection to this view raised by Joseph (:04, p. 24) that the photo-receptors of amphioxus do not occur in the exact region from which the lateral eyes may have arisen does not appear to me to be really serious.

The steps whereby the lateral eyes have come into existence are by no means easily retraced, and it is for this very reason that any indication such as that afforded by amphioxus is of the utmost importance. Whatever has been the exact course followed by the eye in its differentiation, two remarkable but well-recognized features have resulted; first, the retinal elements of the lateral eyes are inverted in relation to the stimulus as compared with the great majority of sense organs, and, secondly, the retina in vertebrates develops not directly from the external ectoderm, but as an outgrowth from the brain. *It is rather striking that two investigators have published, apparently quite independently, essentially the same explanation of these facts. Balfour ('85, p. 508) long ago pointed out that, if we imagine that the retinal part of the lateral eye was involved in the infolding that gave rise to the central nervous organs, then the final positions of the rods and cones at the surface of the retina away from the light would be satisfactorily explained, for this surface is the morphologically external surface of the ectoderm. This explanation assumes that the eye was

functional on the exterior of the vertebrate ancestor before this animal had an infolded central nervous system, and that in the course of its differentiation it had passed as a functional eye into the deeper parts of the head and out to the surface again, a process not so difficult to understand when it is kept in mind that the bodies of many tunicates and of amphioxus are relatively transparent. Essentially the same explanation has been brought forward recently by Jelgersma (:**06**), who believes that the eye in its transition between its supposed place of origin in the skin and its final position in the vertebrate head is well represented by the eye of the larval tunicates. Boveri (:**04**) has called attention to the strong probability that the lateral eye has been derived from photoreceptors in the central nervous system, and has pointed out that the eye-cups of amphioxus are the probable source. He has not, however, attempted to trace these eye-cups back, as Jelgersma (:**06**, p. 393) has done, to a possible origin in the skin, but implies that they may have arisen in place.

Although I believe that the explanation first advanced by Balfour as to the origin of the lateral eyes of vertebrates has some truth in it, there are certain aspects of it which in view of the present investigations need further consideration. Its first assumption is that the skin of the ancestral vertebrate contained photoreceptors. The fact already mentioned, that the skin of some amphibians and fishes, particularly ammocoetes (Parker, :**03**^{c}, :**05**^{b}), is so supplied, would lead to the expectation that the skin of amphioxus would also contain such organs. My own studies have given no grounds for this belief, and, though I have not been able conclusively to prove the contrary, the evidence seems to favor the idea that the skin of amphioxus is not sensitive to light. As nothing is known, so far as I am aware, of the condition of the skin in this respect in tunicates, adult or young, the belief that the skin of the ancestral vertebrate contained photoreceptors must remain a pure hypothesis, and it is conceivable that the photoreceptors of the vertebrate eye may have arisen, not in the skin before the central nervous system was differentiated, as suggested by Balfour and by Jelgersma, but, as intimated by Boveri, from the cells of the central nervous system itself, in positions much as we find them now in amphioxus.

The assumption of an external origin for the vertebrate photoreceptors is helpful only in that it appears to offer an explanation of the inverted positions of the rods and cones in the vertebrate retina. But this explanation requires that from the time the photoreceptors were formed in the skin till they made a part of an organized retina, they should occupy the morphologically outermost portion of the cellular

layer in which they were imbedded and that the individual photoreceptors should be so oriented that their sensory ends would be toward the morphologically outer surface of this layer and their nervous ends away from it. In amphioxus it is true that the photoreceptors lie near the morphologically outer surface (the surface of the central canal), but their orientation is by no means constant in relation to this surface. In some the sensory ends point toward this surface, but in most such is not the case, and in a few they may even point away from this surface. It therefore seems to me obviously impossible to explain the orientation of the retinal rods and cones as transferred from the skin to the retina through a series of stages in one of which as much freedom of position is shown as among the photoreceptors of amphioxus. Nor, as Metcalf (:**06**, p. 528) has pointed out, is the condition more favorable in the larvae of the tunicates, for here the photoreceptor cluster in the brain is so large compared with the thickness of the cellular wall in which it is imbedded (Froriep, :**06**, p. 145) that its orientation is no more related to the morphologically outer surface of the wall than that of the eye-cups of amphioxus is. For these reasons I believe that the inversion of the vertebrate rods and cones in relation to the light is not due to their origin from definitely oriented external photoreceptors, and since there is no positive evidence of the existence of these receptors in the skins of animals that may fairly represent ancestors of the vertebrates, it seems to me that we are not warranted in assuming their presence at all. I therefore agree with Boveri in believing that the photoreceptors of vertebrates have arisen in the central nervous system and not in the skin, as assumed by Balfour and by Jelgersma.

If the unusual position and orientation of the rods and cones in the vertebrate retina are not due to the origin of these bodies from external photoreceptors, how then are these peculiarities to be accounted for? The position of the photoreceptor near the central canal is due in my opinion to the method of growth of the nerve-tube, for the epithelium surrounding the central canal is the source of the various cells in the wall of the tube. When, therefore, a new type of cell, like the photoreceptor, appears, it would be natural to expect it to arise from this undifferentiated material, and, in my opinion, the photoreceptors of amphioxus and of the tunicate larvae are in their position of origin. This position is retained by their derivatives the rod- and cone-cells.

The very exact orientation of the rods and cones involves factors quite different from those that govern their general position. The eye-cups of amphioxus show only a very slight degree of orientation, but so far as this goes, it is correlated with habit, in that the majority

of the eye-cups are directed ventrally and the animal usually rests in the sand obliquely with the ventral side uppermost. Thus the majority of the eye-cups are in a position to receive effective stimulation. If we imagine the body of amphioxus to be increased in muscular strength, etc., whereby it would approach more nearly the condition in the fishes and would consequently add much to its thickness, it follows that the posterior portion would become less transparent and the photoreceptors of the anterior end would be the only ones left in position for effective stimulation. With the development of the mouth cavity, the gills, etc., the source of light for the anterior photoreceptors would become chiefly lateral and dorsal, and their orientation would doubtless conform to this plan of illumination. If in accordance with this scheme each eye-cup assumed the best possible orientation, it would lie with its open end directed laterally and perhaps somewhat dorsally, *i. e.*, the contained sense cell would be oriented with its sensory end away from the light and its nervous end towards this stimulus. With the disappearance of the surrounding pigment cells as the cluster of photoreceptors became a single retina, these elements would be oriented as the rods and cones are. It is in this way, I believe, that the rods and cones of the vertebrate eye have become inverted, rather than that the inversion is inherited from a condition on the external surface of the body.

Not only may the rod- and cone-cells be thus oriented at the beginning, but it seems to me that their subsequent relations to the surrounding parts tend to keep them so. The chief factor in this respect is the supply of materials necessary for their activity. Directed as they are away from the central dioptric part of the eye, their sensory ends, which are the parts most quickly exhausted by activity, are turned toward the chief blood-supply, the choroid layer of the eye, and are, therefore, in a most advantageous position to receive new materials for metabolism. That important substances reach them from this side is seen in the fact, well attested by experiment, that if the retinal pigment layer is removed from a live retina, the regeneration of the visual purple in the rods is much retarded, if not completely stopped, though simply placing the layer back again upon the retina will cause this process to be resumed. Thus the inverted position of the rod- and cone-cells is the one best adapted to keep their most easily exhausted parts nearest the supply of materials necessary for their activities and still hold them open to access to light. This factor is doubtless one that has tended to retain the rod- and cone-cells in their inverted positions.

The condition of light receptors in amphioxus lends no support to

such views of the origin of the lateral eyes of vertebrates as have been advanced by Sharp (**'85**), Burckhardt (:**02**), and others, according to which the lens is regarded as having been derived from the primitive retina, now replaced by a photoreceptive differentiation of a deeper ganglionic part. I agree with Boveri in looking upon the eye-cups of amphioxus and, I may also add, the corresponding elements of the tunicate eye as the forerunners of the vertebrate retina, and, though I was at first inclined to ascribe to these a direct origin from the external skin, I now believe that we at least have no good reason for this assumption.

The chemical sense is the only one in amphioxus that seems to possess a well-marked special organ, the so-called olfactory pit, and yet for this organ both Nagel's experiments and mine gave no signs of sensitiveness other than that which characterizes the skin of the anterior end. Notwithstanding this negative evidence, the morphological relations of this pit are such that I believe it is very probably the homologue of the olfactory organ of the higher vertebrates. That a special function has not been discovered for the olfactory pit in amphioxus is perhaps not surprising when it is remembered that no direct physiological evidence whatsoever is at hand bearing on the function of the olfactory organs of fishes. That these organs are undoubtedly of great significance in the life of a fish is attested by the extent of their surfaces and by the size of the connected parts of the brain, and yet, so far as the habits of fishes are concerned, we have no conclusive evidence as to their real uses.

The outer surface of amphioxus is sensitive to a variety of substances, such as nitric acid, picric acid, alcohol, etc., and to all these substances the animal responds by withdrawing. Nothing could be discovered about its reactions that could lead to the belief that the chemical sense was connected with feeding. This sensitiveness was found in amphioxus to be dependent, not upon nerves from the region of the mouth that had invaded the outer skin, as Herrick (:**03**) has shown for many fishes, but upon the segmental nerves of the region stimulated, for the posterior third of an amphioxus will react, like the whole animal, to effective chemical stimuli. The chemical sense of amphioxus is, then, not especially associated with its mouth or its feeding habits, but is a general integumentary sense, the function of which seems to be to help the animal to escape an unfavorable chemical environment. Apparently this is the primitive function of the chemical sense as it is met with in the skins of many animals, and this unspecialized sense has afforded a basis from which in the region of the mouth the specialized senses of smell and taste (both of which are

chiefly concerned with food discrimination) have been differentiated. This unspecialized chemical sense has been retained in the skin of the frog and other amphibians and in the irritable mucous surfaces of the higher vertebrates, but its chief representatives in the higher forms are its derivatives, the senses of taste and of smell. Of these, amphioxus possibly possesses the sense of smell.

Amphioxus may, therefore, be said to be an animal that possesses *in potentia* at least the sense organs of the vertebrates. Its outer surface is provided with tactile organs, but it does not possess the derivatives of these, the lateral-line organs and the ear. Its outer surface also contains undifferentiated chemical sense organs, but it cannot be said to have a sense of taste, and the only evidence of a sense of smell is morphological. Its outer surface, like that of the higher vertebrates, contains temperature organs. Amphioxus also has in the walls of its nerve-tube photoreceptors, which may well be the forerunners of the rod- and cone-cells of the vertebrate retina. It is thus an animal of fundamental importance for the understanding of the vertebrate sense organs.

9. Summary.

1. Amphioxus is only very slightly sensitive to light.

2. It responds to a rapid increase of light, but not to a rapid decrease.

3. The only known photoreceptors in amphioxus are the eye-cups in the wall of the nerve-tube.

4. Amphioxus is photokinetic and negatively phototropic.

5. Amphioxus is stimulated by water warmer than that in which it lives (31° C.) and is killed in water at 40° C. or higher.

6. It is also stimulated by water colder than 31° C. and is killed by lengthy exposure to water of 4° C. or lower.

7. It is thermokinetic and negatively thermotropic.

8. The outer surface of amphioxus, especially the oral hood and the tentacular cirri, is sensitive to mechanical stimuli.

9. Amphioxus is also sensitive to sound vibrations.

10. It is thigmotropic, and slightly rheotropic and geotropic.

11. The outer surface of amphioxus is sensitive to solutions of nitric acid, potassic hydrate, picric acid, alcohol, and to strong ether, chloroform, turpentine, oil of bergamot, and oil of rosemary, but not to solutions of sugar. It is also stimulated by diluted sea water and by fresh water.

12. Amphioxus is negatively chemotropic.

13. The photoreceptors in amphioxus are anatomically distinct from the receptors for thermal, mechanical, and chemical stimuli, and these three are at least physiologically distinct one from another.

14. To all stimuli that induce locomotion amphioxus responds by forward movements when the stimuli are applied to the tail, and by backward movements when they are applied to the middle or to the anterior end.

15. Amphioxus generally buries itself tail first, and in all probability usually swims tail first, though it may reverse both processes.

16. When amphioxus is cut in two, both halves lose much in sensitiveness, the posterior proportionally much more than the anterior. The anterior part of the nerve-tube is brain-like, the posterior part cord-like.

17. The skin of amphioxus contains tactile organs, but amphioxus possesses no derived organs such as lateral-line organs and ears.

18. The photoreceptors of amphioxus are the eye-cups of the nerve-tube, and these probably represent the elements from which the rod- and cone-cells of the lateral eyes of vertebrates have been derived.

19. The rod- and cone-cells of the vertebrate retina are inverted, not because they have retained a morphological position dependent upon an external origin, but because of their orientation acquired as effective eye-cups in the nerve-tube of a primitive vertebrate.

20. The chemical sense organs of amphioxus are located in the skin and are chiefly important as organs for testing the character of the chemical environment rather than for the selection of food. From these undifferentiated chemical sense organs have probably been derived the organs of taste and smell, of which the former are apparrently not present in amphioxus and the latter may be represented by the so-called olfactory pit.

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